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Morphometric analysis of caryopses in nine species of *Eragrostis* (Poaceae) from India using SEM and light microscopy

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Abstract

Seed exomorphic characters of nine different species of *Eragrostis* were investigated by Light and Scanning electron microscopy. In the present study the micro-morphological characteristic features of caryopses such as shape, dimension, colour, epidermal cell surface structure and features of anticlinal and periclinal walls were examined. Light microscopy revealed that most of the studied caryopses varied in shape from obloid to ovoid. The caryopses in most of the species of *Eragrostis* are sticky in nature due to the presence of surface slime cells, which makes them appear shiny and transparent. This morphological feature was able to be observed under SEM but not light microscopy. The nine different species could be differentiated on the basis of shape and position of the hilum and embryo.

Introduction

Seeds provide numerous morphological characters and can be used for taxonomic purposes. Heywood and Davis (1963) emphasized that the use of seed characters can be reliable and constant within taxa. Grass seed morphological features and surface patterns have been used in many studies to identify and compare taxa and genera (Hillman 1916; Jensen 1957; Bogdan 1965; Banerjee *et al.* 1981; Colledge 1988; Lazarides 1997; Matsutani 1986; Nesbitt 2006; Terrel and Peterson 1993; Peterson and Sánchez Vega 2007).

External features of seeds and small fruits tend to be neglected in Floras, and even in detailed taxonomic studies, which is surprising in view of the stability and high systematic value of external characters (Lawrence 1951; Barthlott 1981, 1984).

Seed characteristics, particularly exomorphic features, revealed through scanning electron microscopy have been used in resolving problems of systematics (Karihaloo and Malik 1994; Koul et al. 2000) and evolutionary relationships (Segarra and Mateu 2002). Micromorphological seed characters using a scanning electron microscope (SEM) are relatively consistent across plant species and may thus prove useful in distinguishing different species as well as their grouping under definitive categories (Agrawal 1984; Hoagland and Paul 1978). Few SEM studies have been concerned with the fine structural differences in taxonomic and morphological features of closely related species, especially within groups of plants of the same species (Liu et al. 2005; Joshi et al. 2008).

The Tribe Eragrostideae (Poaceae) contains 80 genera and 1000 species (*sensu* Clayton and Renoize 1986; Conert 1992). Amongst all the grass species currently recognized in the tribe, about 67% are contained in the three largest genera *Eragrostis* (350 species), *Muhlenbergia* (160 species) and *Sporobolus* (160 species) (Peterson et al. 1997). A newer classification based on DNA phylogenies includes about 20 genera in Eragrostideae, with three subtribes (Peterson et al. 2010, 2010a, 2011, 2012) and excludes *Muhlenbergia* and *Sporobolus* that have been moved to Cynodonteae and Zoysieae, respectively (Peterson et al. 2010b).

Eragrostis is used as livestock fodder and the caryopses appear to be of extremely high nutritional value (Ingram and Doyle 2003). For example, *Eragrostis tef* (Zucc.) Trotter (Zuccagni) is used for the production of the traditional breads across the Horn of Africa: Ethiopian *injera* and Somalian *laxoox*, are grown as a crop of commercial importance; *E. clelandii* S.T.Blake and *E. tremula* Hochst. ex Steud. are used as famine foods in Australia and Chad, respectively. Other species, such as *E. tenellus*, are used as ornamental plants, while *E. cynosuroides* (Retz.) P.Beauv. is used in the puja rites in the Hindu temple at Karighatta. Bahia lovegrass (*E. bahiensis* Schrad. ex Schult.) is known as a hyperaccumulator of Caesium-137 and can be grown to remove these highly toxic and radioactive atoms from the environment. Weeping lovegrass (*E. curvula* (Schrad.) Nees) has been planted extensively to combat soil erosion. Tef (*E. tef*) and several other *Eragrostis* species have been introduced to many other African countries, India, the United States of America, and Australia, mainly as specialty foods and forage crops (Ayele et al. 1996; Zeller 2003; Yu et al. 2000, 2006). In *Eragrostis*, typically caryopses have a thin pericarp consisting of a single cell layer, which completely adheres to the seed coat (Jackman 1999; Boechat et al. 2000, 2003). In the grasslands of the northwestern Indian state of Gujarat there are 14 species of *Eragrostis* that have been previously recorded (Shah 1978). These grasslands consist an area of ~1400 km^2 across the Kachh, Saurashtra and Panchmahal districts. The current study examines the macro- and micromorphological characters of caryopses and provides a taxonomic key for the identification of the nine *Eragrostis* species of the Saurashtra and Panchmahal districts.

Materials and Methods

Voucher specimens of the nine species of *Eragrostis* used in our analyses were collected from grassland and forest areas of Gujarat (Blatter and McCann 1936) (Table 1). Specimens are deposited in BARO Herbarium (Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, India). Specimens were identified to species level with comparison to know specimens at The Blatter Herbarium (BLAT) (Table 1). Mature dried caryopses were manually separated from spikelets (15–20 per species) and prepared for light microscopy and SEM study. Light microscopy measurements were made on the mature, dry seeds and diagnostic features photographed using a Stereo Microscope (Olympus microscope-SZ2-ILST).

All the morphometric measurements are averages (n=15–20) and were carried out as per Nesbitt (2006). Length of caryopses (L) was measured (in mm) parallel to the middle vertical axis included embryo tip, either in dorsal or ventral view. Breadth of caryopses (B) was the maximum width (in mm) on the horizontal axis measured either in dorsal or ventral view. Thickness of caryopses (T) was the maximum width (in mm) measured at right angles to the breadth and in the same horizontal plane, such that T≤B. The length to breadth ratio (L:B) was calculated as the length of caryopses divided by breadth and multiplied by 10. The thickness to breadth ratio (T:B) was calculated as the thickness of caryopses divided by breadth and multiplied by 100. The length of the embryo (from embryo tip to scutellum/endosperm boundary) was calculated as a % of caryopses length (Embryo %). Hilum % was calculated as the length of the hilum for linear hila (measured from base to tip) and for basal and subbasal hila (from base of caryopses to end of hilum) and calculated as a % of

Table: 1 List of *Eragrostis* species studied.

Botanical name	Blatter Herbaria No.
Eragrostis cilianensis (All.) Vignolo ex Janch	84583
Eragrostis ciliaris (L.) R.Br.	84506
Eragrostis japonica (Thunb.) Trin.	84802
Eragrostis nutans (Retz.) Nees.	84889
Eragrostis pilosa (L.) P.Beauv	84895
Eragrostis tenella (L.) P.Beauv. ex Roem & Schult.	85044
Eragrostis tremula (Lam.) Hochst. ex Steud.	85060
Eragrostis unioloides Nees ex Steud.	85079
Eragrostis viscosa (Retz.) Trin.	85205

caryopses length. All the dimensions details are presented in Table 2 and a summary of diagnostic characters are presented in Table 3.

To obtain SEM images, samples were attached to carbon conducting tape and mounted onto brass stubs. Seeds were washed with absolute alcohol or acetone for 1–2 mins to remove any debris, dried and placed on the stub with dorsal, ventral and lateral side facing upwards, and photographed on JEOL JEM – 5610 SEM with a voltage of 15KV. The observed features are presented in Table 4.

Results and Discussion

Across the nine species studied caryopses exhibited great variation in micromorphological surface patterns. *Eragrostis pilosa* had the largest caryopses 0.7 mm long (L), breadth (B) 0.3 mm and thickness (T) 0.4 mm whereas *E. ciliaris*, *E. tremula* and *E. viscosa* had the smallest length of 0.4 mm. *Eragrostis unioloides* has a maximum L:B ratio (35.51) and T:B ratio (204.66), while *E. cilianensis* has a minimum L:B ratio (12.25) and *E. tenella* has a minimum T:B ratio (96.33). *Eragrostis viscosa* exhibits the maximum embryo (64.08%) and hilum (24.46%) percentage in the caryopses, whereas *E. japonica* has a minimum embryo percentage (31.91%), and *E. tenella* has a minimum hilum percentage (11.69%).

The colour of mature caryopses varied from light to dark brown with a smooth shining surface. Shape of the caryopses in *E. japonica*, *E. nutans*, *E. tenella*, *E. unioloides* and *E. viscosa* varied from obloid to ovoid. When viewed dorsally *Eragrostis tenella* and *E. viscosa* had a slightly rounded apex compared to *E. japonica* and *E. nutans*. Caryopses of *E. tenella* have an almost equal breadth and thickness hence the minimum T:B ratio (96.33). A common feature noted was the absence of the ventral groove/sulcus on the caryopses. Apart from this, dorsal/lateral striations were present only in *E. cilianensis*, *E. tremula* and *E. unioloides* (Fig. 1), whereas other species had no striations. Of the nine species studied, the caryopses of *E. pilosa*, *E. tremula* and *E. unioloides* (Fig. 1c, u, x) were compressed laterally while caryopses of the others were ±terete. Peterson and Sanchez (2007) reported *E. japonica*, *E. pilosa* and *E. ciliaris* as ±dorsally compressed; however, we found that *E. ciliaris* and *E. japonica* were terete but *E. pilosa* was laterally compressed.

Caryopses in most of the nine species were sticky (Kreitschitz et al. 2009) because of the presence of surface slime cells, giving the surfaces a shiny and translucent appearance that was difficult to observe under a light microscope but very apparent under SEM.

Table: 2 Dimensional details of caryopses of species of *Eragrostis*

L = Length; B = Breadth; T = Thickness; L:B = (Length/Breadth)×10; T:B = (Thickness/Breadth)×100; Embryo% = length of embryo as a percentage of total length of caryopsis; Hilum% = length of hilum as a percentage of total length of caryopsis

Species Name	Size (mm)						
	L	B	T	L:B ratio	T:B ratio	Embryo %	Hilum %
E. cilianensis	0.5±0.01	0.4±0.02	0.4±0.02	12.25±0.55	102.32±2.83	35.58±6.24	17.73±1.11
E. ciliaris	0.4±0.01	0.2±0.02	0.2±0.01	18.45±0.72	100.70±5.32	38.88±5.43	17.83± 1.50
E. japonica	0.5±0.01	0.2±0.01	0.2±0.01	20.46±0.79	106.89±4.45	31.91±6.38	15.30±2.82
E. nutans	0.5±0.01	0.2±0.02	0.2±0.02	22.34±2.16	98.64±11.58	45.64±5.28	14.57±0.64
E.pilosa	0.7±0.05	0.3±0.01	0.4±0.01	21.50±1.70	120.89±5.05	61.75±5.77	13.33±1.60
E. tenella	0.5±0.02	0.2±0.02	0.2±0.02	18.19±1.39	96.33±9.81	47.82±3.34	11.69±2.03
E. tremula	0.4±0.03	0.3±0.02	0.4±0.02	14.48±1.42	131.36±9.38	49.82±5.76	13.46±0.78
E. unioloides	0.7±0.04	0.2±0.01	0.4±0.03	35.51±3.67	204.66±17.35	48.76±4.35	12.55±1.86
E. viscosa	0.4±0.01	0.2±0.01	0.2±0.01	17.33±0.86	117.20±6.85	64.08±15.37	24.46±3.38

Fig.1. Light Microscopic features of caryopses of Eragrostis **a–c,** *Eragrostis cilianensis*. **d–f,** *E. ciliaris*. **g–i,** *E. japonica*. **j–l,** *Eragrostis nutans*. **m–o,** *Eragrostis pilosa*. **p–r,** *E. tenella*. **s–u,** *E. tremula*. **v–x,** *E. unioloides*. **y–a1:** *E. viscosa*. Column views: **a, d, g, j, m, p, s, v, y,** dorsal surface; **b, e, h, k, n, q, t, w, z,** ventral surface; **c, f, i, l, o, r, u, x, a1,** lateral surface.

Table 3: Light microscopic features of caryopses of species of *Eragrostis*

Embryo class: Large ≥ 46%, Short ≤ 45%

Species Name	Shape	Colour	Texture	Compressions	Dorsal/ Lateral striations	Scutellum shape	Embryo Class	Distinct features observed
E. cilianensis	Orbicular	Dark brown to black	Smooth, shiny	Not compressed	Present	Sickle-shape	Short	Embryo occupies very small portion of caryopses.
E. ciliaris	Obloid to obovoid	Dark brown	Smooth, shiny	Not compressed	Absent	V-shaped	Short	Towards the proximal end white color spot is seen, near the hilum
E. japonica	Obloid	Dark brown	Smooth, shiny	Not compressed	Absent	V-shaped	Short	On ventral surface longitudinal lines are seen which are prominent below the hilum.
E. nutans	Obloid to ovoid	Dark brown	Smooth, shiny	Not compressed	Absent	V-shaped	Short	Scutellum is more concave than *Eragrostis japonica.*
E. pilosa	Ovoid to obloid	Dark brown	Smooth	Laterally compressed	Present	V-shaped	Large	On ventral side longitudinal lines are present
E. tenella	Obloid to obovoid	Dark brown	Smooth, shiny	Not compressed	Absent	V-shaped	Large	Caryopses are translucent.
E. tremula	Globular to obloid	Creamish to light brown	Smooth, shiny	Laterally compressed	Present	Sickle-shape	Large	Towards the proximal end minute dotted slimy glands are present
E. unioloides	Ovoid	Brown to dark brown	Smooth, shiny	Laterally compressed	Present	V-shaped	Large	Scutellum is divided into two parts by embryo axis, which is continuous with the surface.
E. viscosa	Obloid to ovoid	Dark brown	Smooth, shiny	Not compressed	Absent	V-shaped	Large	Surface is more shiny than for other species of *Eragrostis.*

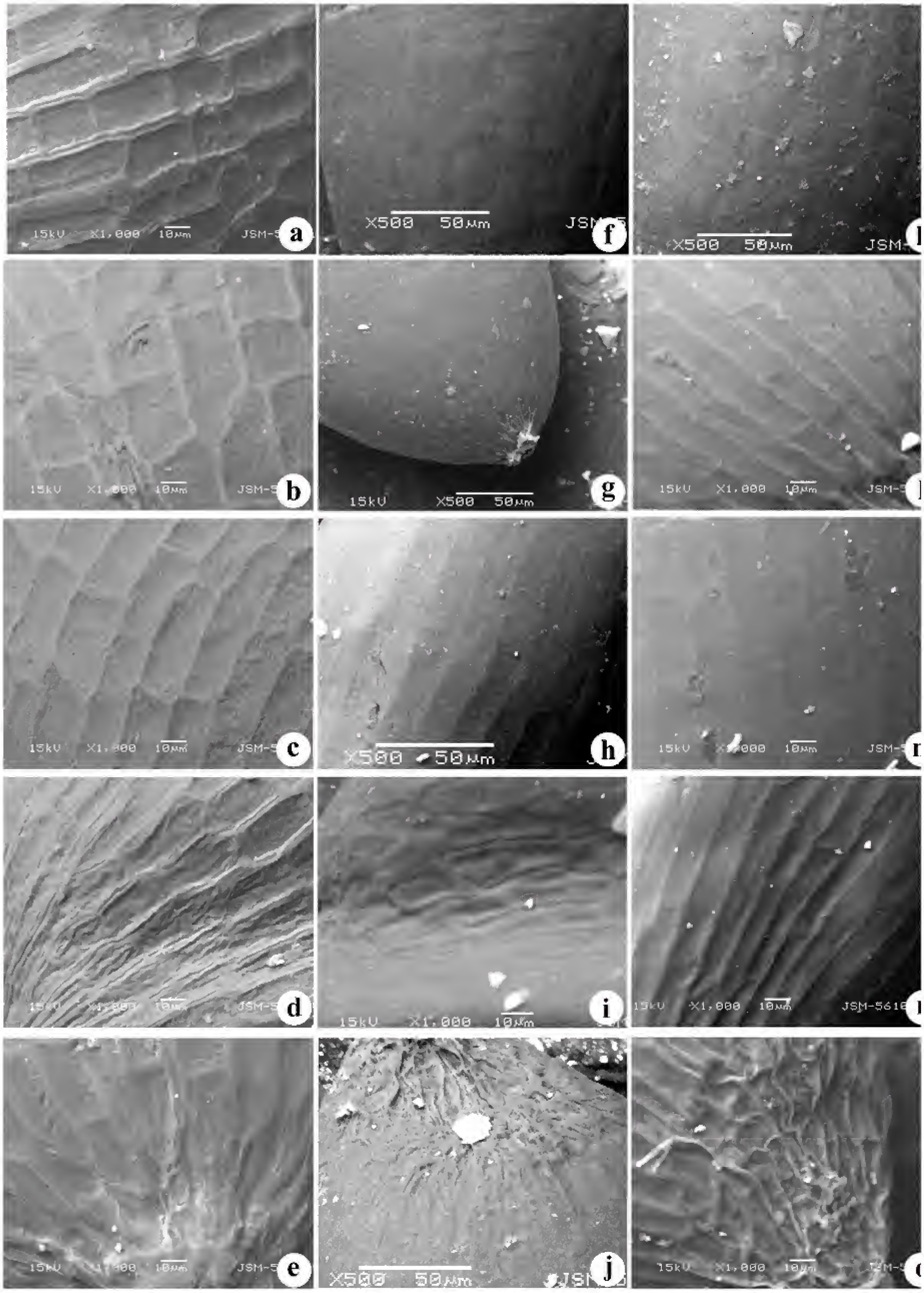

Fig 2: Scanning Electron Microscopic features of caryopses of *Eragrostis* **a–e,** *Eragrostis cilianensis*. **f–j,** *E. ciliaris*. **k–o,** *E. japonica*. Column views: **a, f, k,** dorsal surface; **b, g, l,** ventral surface; **c, h, m,** lateral surface; **d, i, n,** embryo surface; **e, j, o,** hilum surface.

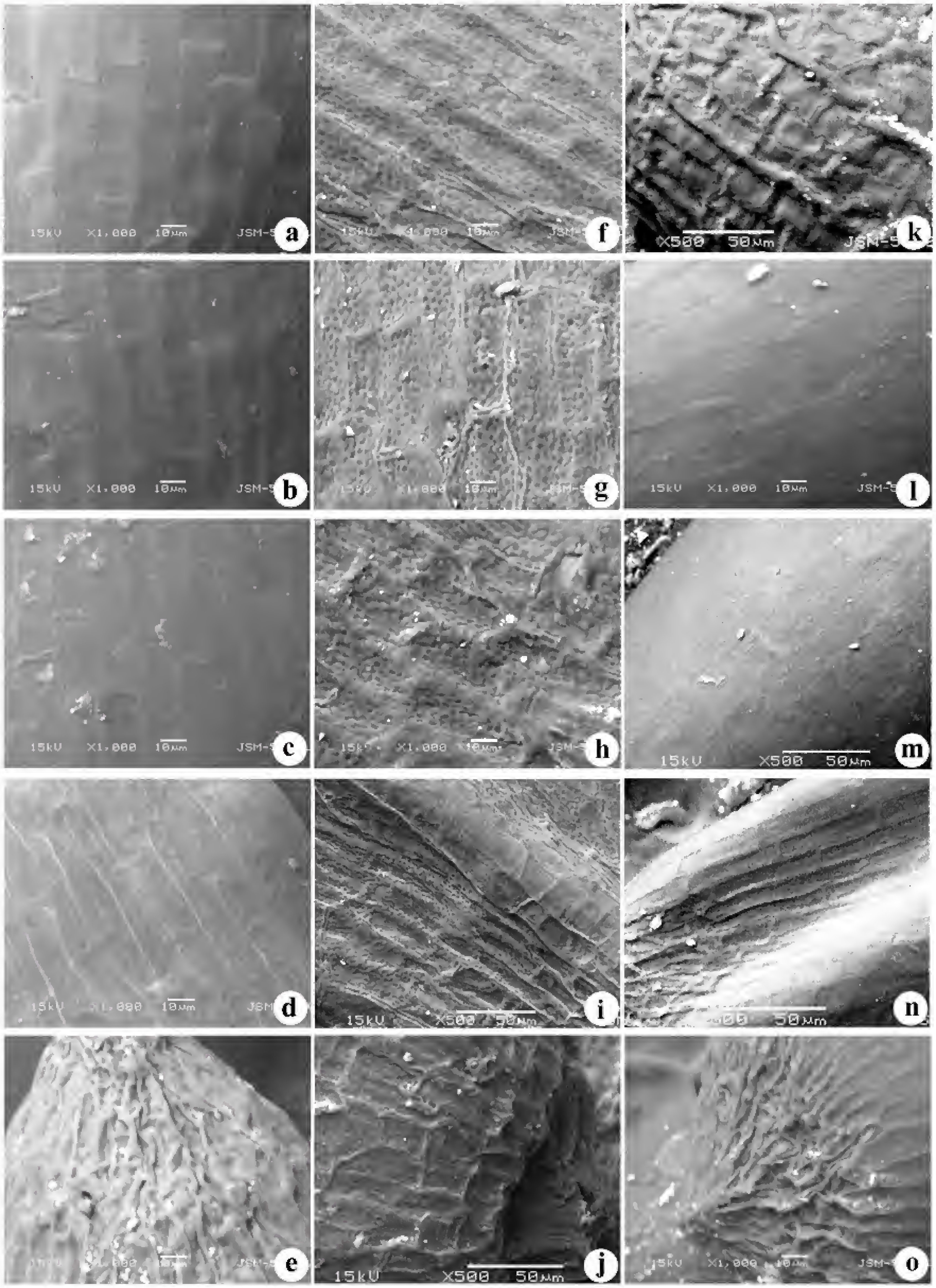

Fig 3. Scanning Electron Microscopic features of caryopses of *Eragrostis* **a–e**, *Eragrostis nutans*. **f–j**, *E. pilosa*. **k–o**, *E. tenella*. Column views: **a, f, k,** dorsal surface; **b, g, l,** ventral surface; **c, h, m,** lateral surface; **d, i, n,** embryo surface; **e, j, o,** hilum surface.

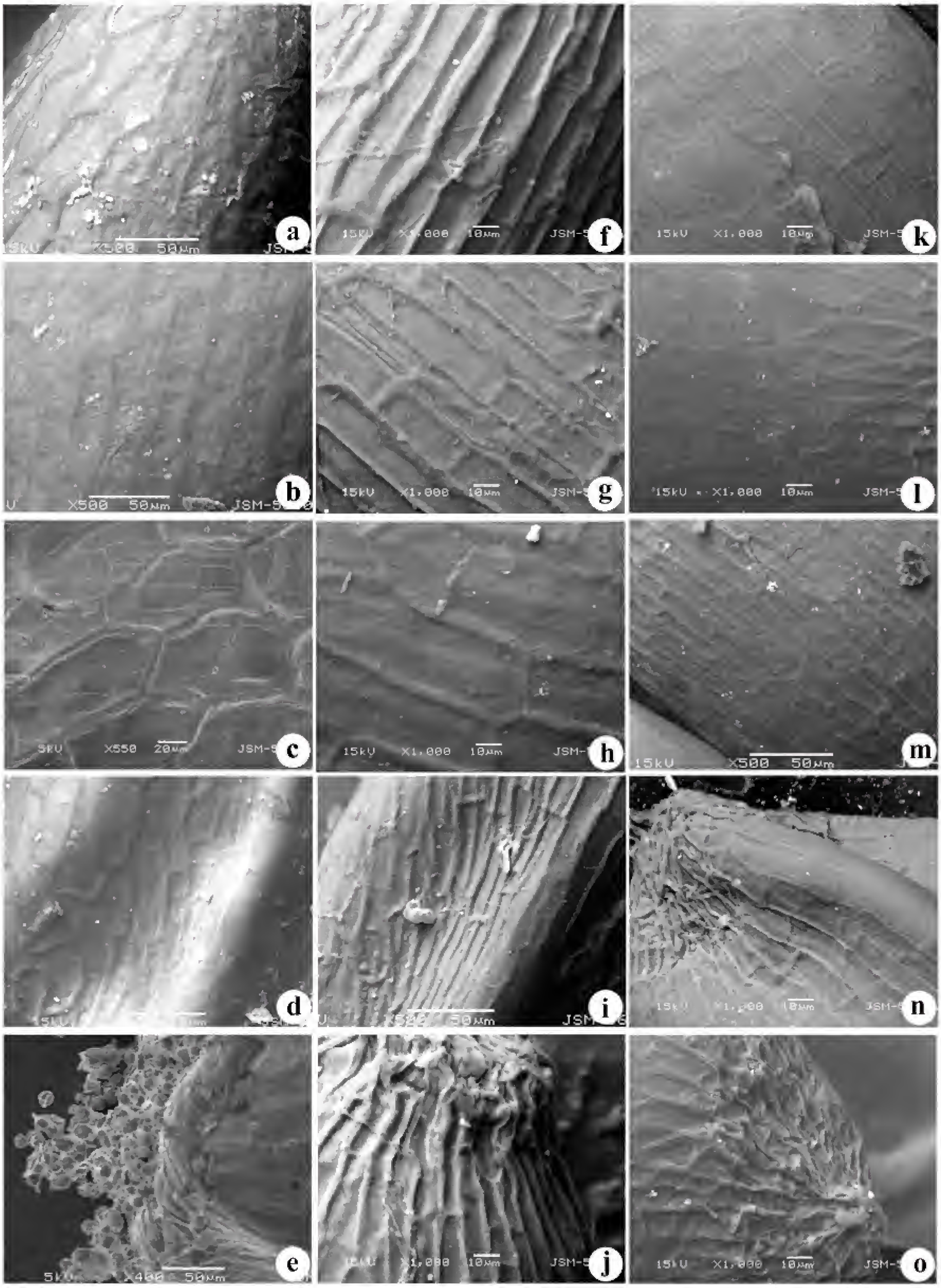

Fig 4. Scanning Electron Microscopic features of caryopses of *Eragrostis* **a–e**, *Eragrostis tremula*. **f–j**, *E. unioloides*. **k–o**, *E. viscosa*. Column views: **a, f, k,** dorsal surface; **b, g, l,** ventral surface; **c, h, m,** lateral surface; **d, i, n,** embryo surface; **e, j, o,** hilum surface.

The shape and surface features of the axis and scutellum separated the embryo into two different types. *Eragrostis cilianensis* and *E. tremula* both had a sickle-shaped scutellum (Fig. 1a, s) whereas all other species had a 'V'-shaped scutellum. The axis surface of *E. ciliaris* and *E. tremula* was glabrous, whereas in *E. cilianensis, E. japonica, E. nutans, E. tenella, E. unioloides* and *E. viscosa* the axis and scutellum surface showed reticulate architecture.

The ventral surface of *E. ciliaris, E. tremula* and *E. unioloides* showed rectangular to polygonal areas whereas in *E. ciliaris* (Fig. 2g) and *E. tenella* (Fig. 3m) it was smooth and homogeneous. *Eragrostis japonica, E. nutans* and *E. viscosa* had superficial architecture which was shallow and prominent. Dimensional details of the observed features are presented in Table 5. Tissue towards the proximal end of the hilum appears highly convoluted. *Eragrostis pilosa* showed a contrasting feature in having a reticulate-foveate surface with thick rugae (Fig. 3f, g). Two unique diagnostic features were observed in *E. tremula*, namely superimposed rows of reticulum were present on the lateral surface (Fig. 4c) and globular slimy glands were present at the proximal end on ventral surfaces (Fig. 4e). The upper reticulum was pentagonal to hexagonal with a smooth, thick, and elevated tangential wall in *E. tremula*, while the reticulum was elongated rectangular with smooth thin undulating walls in *E. pilosa*. The striations on the dorsal surface of *E. pilosa* had a maximum length (79.56 µm) among all studied specimens while the striations on the ventral surface of *E. nutans* had a maximum length (71.78 µm) and on the lateral surface *E. tremula* had a maximum length (92.45 µm). Other dimensional details are represented in Table 5.

Our study of epidermal surfaces reveals a number of important micromorphological characters exhibiting interspecific variation that are useful for identification.

Table 5. Dimensional features of striations (under SEM) on surface of caryopses of *Eragrostis*

Species Name	Dorsal surface		Ventral surface		Lateral surface	
	L (µm)	B (µm)	L (µm)	B (µm)	L (µm)	B (µm)
E. cilianensis	26.73±2.85	16.54±1.50	34.46±7.56	21.27±1.38	41.27±4.39	16.54±0.76
E. ciliaris	–	–	–	–	–	–
E. japonica	–	–	60.54±3.50	14.56±1.45	56±1.04	13.10±0.35
E. nutans	71.81±7.86	14.23±0.93	71.78±4.62	15.12±0.61	66.23±2.89	11.56±1.27
E. pilosa	79.56±1.86	19.11±0.93	51.92±1.30	17.56±0.93	42.45±6.25	18.45±1.27
E. tenella	42.48±1.79	20.05±1.63	–	–	–	–
E. tremula	73.48±8.47	30.86±1.82	–	–	92.45±6.59	46.23±1.86
E. unioloides	70.95±3.12	13.26±1.76	58.74±6.51	14.94±0.88	78.50±3.24	25.75±2.59
E. viscosa	55.69±1.85	12.16±0.99	–	–	47.80±7.33	8.52±1.70

Key to species of Eragrostis based on seed characteristics

1a. Caryopses not compressed 2
1b. Caryopses laterally compressed 7

2a. Scutellum sickle-shaped .... *E. cilianensis*
2b. Scutellum V-shaped 3

3a. Caryopses strictly obloid .... *E. japonica*
3b. Caryopses obloid to obovoid to ovoid 4

4a. Dorsal surface smooth (under SEM) .... *E. ciliaris*
4b. Dorsal surface with reticulate pattern (under SEM) 5

5a. Lateral surface smooth (under SEM) .... *E. tenella*
5b. Lateral surface with reticulate pattern (under SEM) 6

6a. Embryo short (embryo length ≤45% of caryopsis) .... *E. nutans*
6b. Embryo large (embryo length ≥46% of caryopsis) .... *E. viscosa*

7a. Scutellum sickle-shaped .... *E. tremula*
7b. Scutellum V-shaped 8

8a. Caryopses surface rectangular-foveate with pits (under SEM) .... *E. pilosa*
8b. Caryopses surface rectangular and smooth (under SEM) .... *E. unioloides*

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